STUDIES ON THE GOITROGENIC INFLUENCE OF COW'S MILK ON MAN

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SERIES A. II. CHEMICA

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Studies on the goitrogenic influence of cow's milk on man

Both in this country[1,2] and abroad[3,4] some evidence has been reported in the recent literature in favour of the hypothesis that goitrogenic activity may be transferred by way of cow's milk from fodder plants or weeds to man. In their investigations in Australia, Clements and Wishart[3] have found that the milk of cattle fed on large amounts of marrow kale has an inhibitory or even iodine-depleting effect on the thyroid gland in man, whereas milk of cattle fed on other kinds of fodder does not have this effect. These workers believed L-5-vinyl-2-thiooxazolidone (VTO), thiocyanate, or possibly cheirolin to be the active goitrogen.[4] They did not, however, succeed in isolating the active component. In England, Greene and his coworkers[5] have tried to reproduce Clement and Wishart's results. In their experiments the marrow kale milk disturbed the ^{132}I uptake of the thyroid less than did the control milk, the effect of both being very weak, however. In animal experiments in the Netherlands, Van der Veen and Hart[6] found no goitrogenic activity in milk from cattle fed on turnip containing 10 mg/kg of VTO. Peltola[1,2] in this country, has observed that the milk of a certain community had some goitrogenic effect on rats, but that of the neighbouring community had none. The content of goitrogenic substances in the milk or in the blood of the test subjects has not been stated in the above reports. Neither have any data about the iodide content of the milk or body fluids been given.

Since, however, the results of the experiments mentioned above have been cited in the literature as proof of the goitrogenic properties of milk, it was of importance to reinvestigate the problem in the conditions prevailing in Finland, where goitre of the endemic type is common. At the same time we tried to elucidate which substances might be the cause of the disturbance in man observed by the Australian investigators[3,4].

Regarding the occurrence and chemical nature of the goitrogens possibly present in milk, we refer to the recent paper by Virtanen[7] in which the problem is extensively discussed in the light of both the literature and experiments made in this institute.

Experimental

The experiments were performed in accordance with the usual procedure in studies on the effect of antithyroid substances in man[8,9,10] which both Clements and Wishart[3], and Greene *et al.*[5] have also used.

1 to 5 microcuries of ^{131}I (carrier-free) were given to a fasting test subject in the morning. The subject lay down with his head in a mould of foamy plastic, a scintillation crystal was firmly held in a stand immediately in front of the midline of his throat, a little below the thyroid cartilage where the maximal uptake was expected (Fig. 1). The position of the tube was marked on the skin. The activity was measured at least every 15 minutes, three 2-minute periods being counted each time. The count rate was immediately plotted against the square root of time. When at least four successive measurements had shown this graph to be a straight line, the test subject was given 1.5 to 2.2 litres of milk to drink within an hour. The ^{131}I uptake by the thyroid was then followed for the next two and a half hours at least. After this, the latter part of the curve was frequently used for the investigation of the effect of antithyroid drugs or iodide. In this way the sensitivity of the method could be checked.

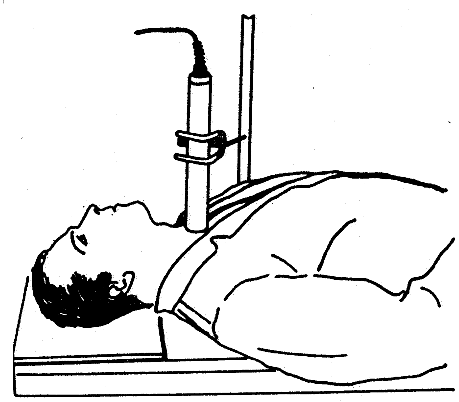

Fig. 1. Counting set up.

Twenty-two healthy adult volunteers, aged 22 to 46, served as test subjects. Seven of them were women, and 15 were men. With some of the subjects the test was repeated after an interval of one month. During the experiment a sample of blood was drawn from the cubital vein of each subject in order to determine the total iodine, protein-bound iodine, and thiocyanate content of the serum.

Fifteen different milk samples were tested (Table 1). Most of these were produced on a feed containing a great excess of plants believed to be goitro-

Table 1

Milk samples used						
Milk	Type of feeding[1])	Amount kg/day/animal	Fat %	I $\mu g/l$	Milk SCN^- mg/l	VTO $\mu g/l$
1. Farm 1 cow 1, 24 Oct.	Schneiders Futterkohl MS grün (SCN^- 5 mg/kg) fresh	30	5.3	15	4.5	0
2. Farm 1 cow 2, 23—24 Oct.	Schneiders Futterkohl MS blau (SCN^- 125 mg/kg) fresh	30	4.8	44	8.0	0
3. Farm 1 mixed milk from 25 cows, 30—31 Oct.	Marrow kale, fresh	25—30	3.4	41	5.9	0
4. Farm 2 3—4 Oct.	Fodder turnip, fresh	36	4.3	11	6.7	12.6
5. Farm 1 cow 1, 13 Oct.	Winter rape (Matador + Lembke 1 : 1)	22—27	3.6	16	3.9	0
6. Farm 3 18—19 Oct.	Winter rape Lembke, fresh	32	3.7	8	8.0	20.3
7. Farm 4 11—12 Dec.	Winter rape Lembke, AIV silage	20	4.8	38	5.1	0
8. Farm 5 8 Dec.	Sugar-beet tops, AIV silage	20	3.8	8	4.4	
9. Farm 6 10 Nov.	Clover-timothy, hay	8—10	3.6	26	0.5	
10. Farm 1 mixed milk from 26 cows a) 28 Nov. b) 5 Dec.	Clover-timothy, AIV silage	26	a) 4.1 b) 4.4	24 72	2.0 2.3	
11. Markret milk, Helsinki a) 11 Oct. b) 30 Nov.			a) 5.0 b) 4.3	28 142	2.4 2.1	0
12. Reconstrued milk, 25% dry matter (Powder from middle Finland 19 Sept.)			27.0*)	126	5.5	
13. Ropy cultured milk (Valio 20 Dec.)			3.9	64	1.5	

[1]) Only the sort of fodder is mentioned which has been suspected to disturb the function of the thyroid gland.

*) % of dry weight.

genic, chiefly *Brassicas*. Several of these milks had an abnormally high thiocyanate content, and two contained small amounts of goitrin (VTO). The milk iodine ranged from 8 to 142 micrograms per litre. Some of the milk samples were used fresh, and some, after deep-freezing, were melted and homogenized. Experiments showed that freezing and storing did not influenced the results.

The protein-bound iodine was precipitated with zinc sulphate in alkaline solution[11]. The method of iodine determination was a modification depending on alkali fusion and cerimetric measurement[11,12]. Thiocyanate was determined by precipitating the proteins with trichloracetic acid and measuring the colour formed with ferric nitrate. L-5-vinyl-2-thiooxazolidone was determined by a spectrophotometric method[13].

Results

a. *Serum iodine and thiocyanate, and* ^{131}I *uptake of the test subjects*

The values for the protein-bound iodine (PBI) of the test subjects were, with one exception, within normal limits (Table 2). The exceptional subject had taken an organic iodine compound as a medicine before the test. The total iodine in the serum of the subjects was remarkably low. In fact, it remained doubtful whether any free iodide at all could be detected with the method used. In those cases in which iodide was administered, however, the expected rise in total iodide values usually followed. The mean PBI value for men was 5.86 ± 0.26 micrograms per 100 ml, and for women 5.21 ± 0.31. The difference (0.65) is not statistically significant. The mean total iodine value for men (6.30 ± 0.33) was higher than that for women (5.45 ± 0.27). This difference (0.85) has some statistical significance ($P < 0.1$). In computing these mean values, the abnormal PBI and the total iodine values for subjects who were given iodide before sampling were omitted.

The thiocyanate values (Table 2) in the serum of the subjects varied between 0.0 and 12.4 mg per litre. The mean for men was 5.13 ± 0.95, and for women 3.39 ± 1.30. The difference between the means for men and women was not significant. It was observed that the thiocyanate content in the serum of smokers (8.73 ± 0.86) was higher than that of non-smokers (2.20 ± 0.28), the difference (6.53) being highly significant ($P < 0.001$). In the iodine and PBI values there was no significant difference between smokers and non-smokers.

When estimating the thyroid activity from the slope (S) of the line (Table 2).

Table 2

Test N:o	F=female M=male	Smoker (+) or not (−)	Dose ^{131}I μC	S	Serum Tot. I (microg/100 ml)	Serum PBI (microg/100 ml)	Serum SCN− mg/l	Milk (Table 1) N:o	Milk (Table 1) Dose	Milk (Table 1) Effect
1	M	−	2.8	160	6.5	6.4	4.1	11 a.	1.5 l	0
2	M	−	1.0	113	6.0	5.9	—	no milk		
3	M	−	2.1	98	7.2	7.0	3.5	4	2.1	0
4	M	−	2.7	38	8.7	7.7	1.6	1	2.0	0
5	M	−	2.4*)	75	13.0	5.4	3.0	3	2.0	0
6	M	−	5.0	137	8.1	6.7	0	2	1.5	0
7	M	−	2.3	62	6.9	6.0	2.2	2	2.0	0
8	M	−	2.8	100	14.0	13.8	1.5	10 b.	2.0	0
9	M	−	2.8	218	4.9	4.9	2.3	8	2.0	0
10	M	+	2.6	80	5.4	—	10.2	11 a.	1.9	0
11	M	+	3.0	125	6.0	5.4	7.0	no milk		
12	M	+	2.3	60	6.8	6.8	12.4	6	2.0	0
13	M	+	4.4	110	5.2	5.1	9.5	5	2.0	0
14	M	+	5.0*)	210	5.6	5.1	7.8	3	1.9	0
15	M	+	2.2	66	—	—	—	no milk		
16	M	+	3.1	184	5.0	5.0	5.4	9	1.9	0
17	M	+	2.5	170	5.2	4.6	6.5	12	1.0	0
18	F	−	2.0*)	—	—	—	—	3	1.5	0
19	F	−	1.8	215	6.3	6.5	1.9	11 b.	1.0	—
20	F	−	2.0	490	5.8	5.9	1.9	10 a.	1.1	0
21	F	−	3.3	400	4.9	4.2	1.0	10 b.	1.5	0
22	F	−	2.0	590	4.8	4.9	3.5	7	1.2	0
23	F	−	2.0	310	4.6	4.7	2.4	7	1.0	0
24	F	−	2.3	460	6.1	5.1	2.0	13	1.0	0
25	F	+	2.1	262	5.6	5.1	11.0	3	2.0	0
26 a.	M	−	2.0	240	6.3	6.3	0			
b.	M	−	2.0	210	6.2	5.4	1.6			
27 a.	M	−	2.0	165	7.4	4.7				
b.	M	+	2.0	124	6.0	3.5				
c.	M	−	2.0	100	8.8	4.9				
d.	M	+	2.0	80	9.1	4.8				

*) ^{131}I given with 2—3 mg ^{127}I

$$S = \frac{C_2 - C_1}{\sqrt{T_2} - \sqrt{T_1}}$$ where C_1 and C_2 are counts/min/μC at times T_1 and T_2

it should be noted that no comparable values can be obtained unless the conditions in the experiments compared are identical. When counts are made at a short distance, as in these experiments, the distance error may be considerable. However, this can hardly explain why the observed slope of the uptake curve was always higher for women than for men (Fig. 2). The mean slope was 122 $\pm$ 13 for men, and 390 $\pm$ 19 for women, the difference between these means being highly significant ($P < 0.001$). Between smokers and non-smokers there was no significant difference in this respect.

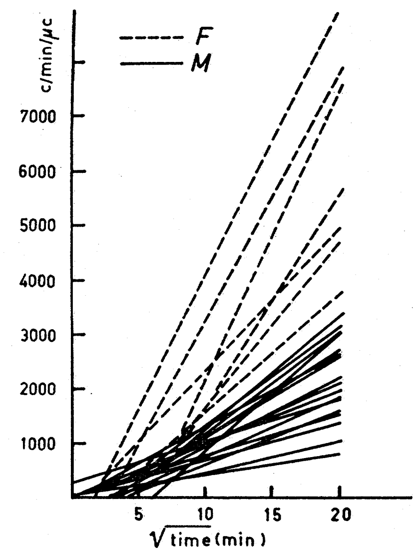

Fig. 2. The relative slopes (S) of the ^{131}I uptake curve in female (F), and male (M) subjects.

b. *Tests with milk samples*

The results of the experiments with milk turned out to be negative. None of the milk samples could be shown to have any effect on the thyroidal accumulation of ^{131}I. Apart from a frequent small fluctuation during the drinking of milk, the thyroidal radioactivity remained a linear function of the square root of time (Fig. 3). (The small fluctuation also occurred when water was drunk: Fig. 3, (17), *cf*[5].) The negative result obtained may be considered significant since not a single exception was found in experiments performed on 22 milk drinkers, and since most of the milk samples were produced on feeds believed to be highly goitrogenic.

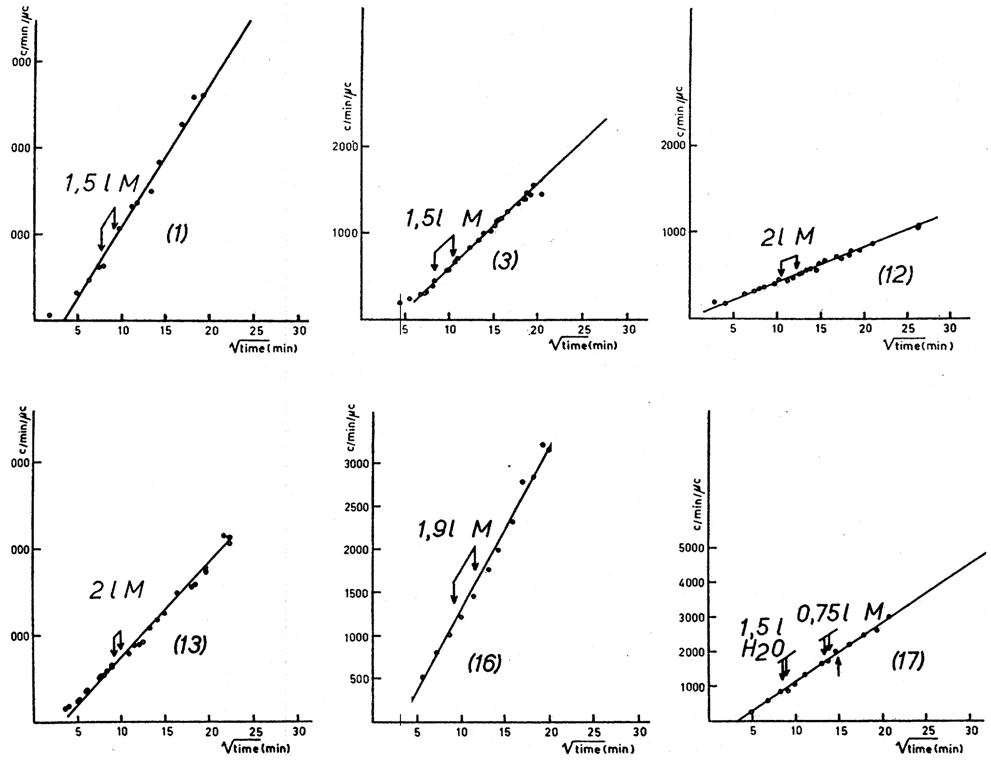

Fig. 3. The effect of the thyroidal ^{131}I uptake curve of milk samples. M = volume of milk in litres. (1) market milk, (3) Turnip milk (high VTO), (12) rape milk (high VTO and SCN^-), (13) rape milk, (16) hay milk, (17) water and milk powder.

c. *Tests with antithyroid compounds and iodide*

The sensitivity of the method to antithyroid substances was of the same magnitude as, or slightly greater than is stated in the literature (Table 3). Thus VTO produced a clearly perceptible thyrostatic effect in a 50 to 75 mg dose (Astwood and his coworkers[14]: 100 mg), thiouracil was active in a 25 mg dose (Stanley and Astwood[8]: 50 mg), and thiocyanate in a 200—400 mg dose (Stanley and Astwood[8]: 1 000 mg). The thyrostatic substances tended to retard or stop the ^{131}I uptake (Fig. 4), but no appreciable depletion of thyroidal ^{131}I could be produced by them.

The only substance in these experiments which caused reduction of thyroidal radioactivity was iodide (Fig. 5), of which a dose of about 1 mg was required to obtain a clear effect (Table 3). When giving 2 to 3 mg of stable iodide as a carrier with ^{131}I, a reduction of thyroidal radioactivity was

Table 3

The effect of neomercazol (NM), thiouracil (TU), L-5-vinyl-2-thio-oxazolidone (VTO), thiocyanate (SCN−), and iodide (I) on the ^{131}I uptake in man.

Test N:o	Substance administered	Dose mg	Effect 0−5	Test N:o	Substance administered	Dose mg	Effect 0−5
22	NM	5	3−4	26	SCN−	181	1
				26	SCN−	205	0
15	TU	25	3	26	SCN−	362	4
13	TU	50	4	26	SCN−	410	3
12	VTO	0.15	0	27	I	0.1	0−1
7	VTO	1.5	0	14	I	0.2	0
6	VTO	20	0	5	I	0.2	2
2	VTO	50	1	27	I	0.3	1−2
25	VTO	50	2	24	I	0.4	0
11	VTO	75	2	27	I	0.6	1−3
19	VTO	75	4	7	I	0.7	0
16	VTO	100	5	4	I	0.7	3
				3	I	1.0	1
10	SCN−	33	0	24	I	4.0	4
23	SCN−	100	2	9	I	6.0	4

0 = no effect
1 = weak or questionable effect
2 = clear effect, no total inhibition
3 = total inhibition less than 4 hours
4 = total inhibition 4 to 24 hours
5 = total inhibition for more than 24 hours

observed during the drinking of milk (Fig. 5 (5)). This happened, though to a smaller degree, even if no milk was drunk (Fig. 5 (18)). Moreover, for some time after the thyroid had recovered from this acute overloading with iodide, a very small amount of iodide, or even the iodide in the milk, was still able to lower the uptake curve.

The effect of small does of iodide (0.1—0.6 mg) was studied in four subjects who received an excess of iodide (0.5—2.0 mg/day) during the six days preceding the test. One person reacted on 0.1 mg, two on 0.3 mg, and three on 0.6 mg (Test 27, Tables 2 and 3). The preceding iodine medication did not alter the ^{131}I uptake curve, although it increased the free iodide in the blood by 3 to 4 micrograms per 100 ml. The sensitivity to small doses of iodide was not significantly increased.

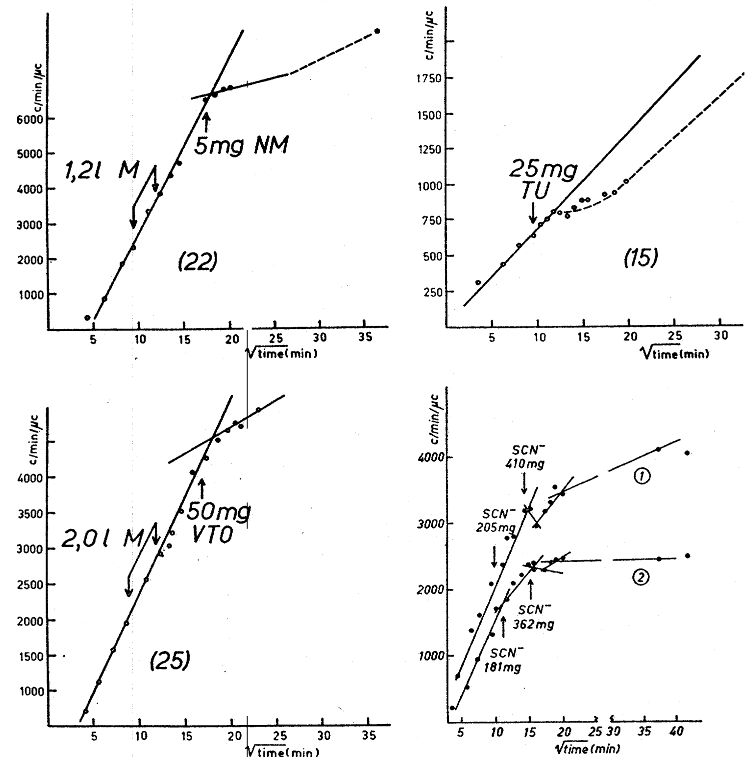

Fig. 4. The effect on the thyroidal ^{131}I uptake curve of neomercazole (22), thiouracil (15), L-5-vinyl-2-thiooxazolidone (25), and thiocyanate (1 & 2).

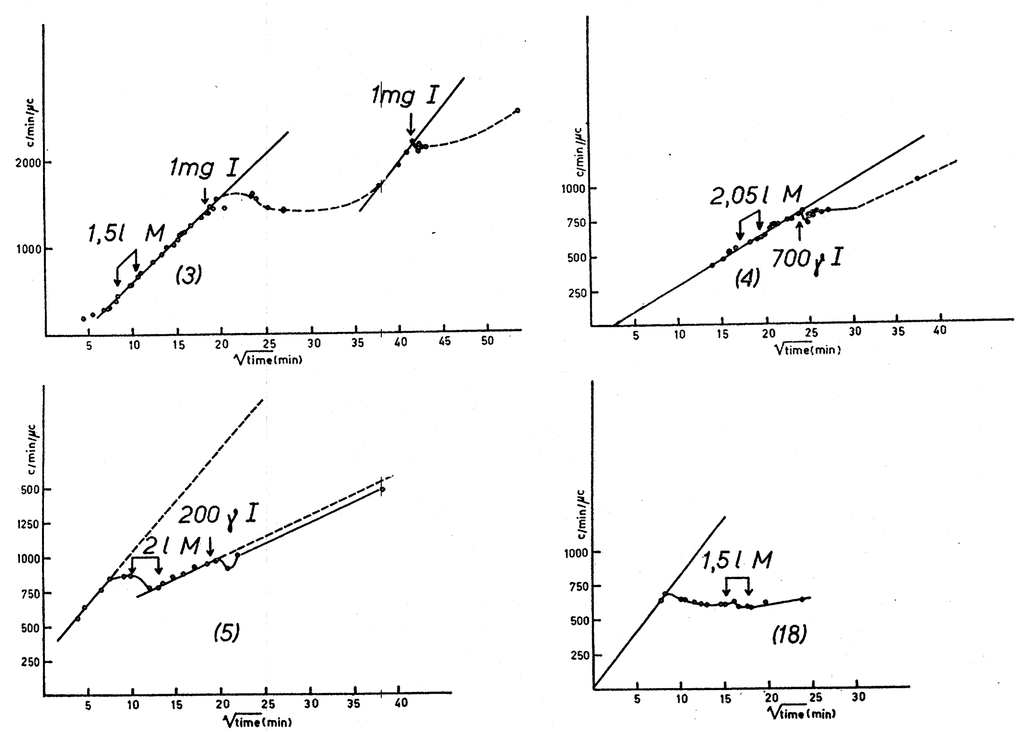

Fig. 5. The effect on the thyroidal ^{131}I curve of iodide. (3) and (4) ^{131}I given without carrier, (5) and (18) 3 mg of ^{127}I given with ^{131}I.

Discussion

It has previously been shown in this institute that although the green parts of *Brassicas* which are most important as cattle fodder, contain appreciable amounts of VTO[13], the VTO content of the milk of cattle fed on these plants cannot exceed 0.1 mg per litre[15]. The thiocyanate content of the corresponding milk hardly exceeds 10 mg per litre[16]. It was likewise shown that no cheirolin or thiourea derived from it can be demonstrated in the milk[7] at all. From the minimum doses shown in this investigation to disturb the accumulation of iodide in the thyroid gland, it appears that ten litres of milk would have to be drunk to obtain the thiocyanate effect, and some hundred litres to obtain the VTO effect. When, in addition, we take into consideration that thiocyanate doses ten times as high are required if the iodine supply is adequate, it is most unlikely that these substances could render milk goitrogenic for man. Nor as these experiments demonstrate, is there any reason to suspect that some unknown factor with goitrogenic effect is present in milk, since no effect could be shown, although most of the milk samples tested were produced by cows on a very *Brassica* diet.

That thiocyanate might exert an effect was rendered still more unlikely by the observation that tobacco smoking increases blood thiocyanate to values far higher than could ever be produced by the amounts of thiocyanate present in milk. Even so, no disturbance, acute or chronic, could be shown in the thyroidal uptake of ^{131}I in smokers.

The effect of iodide on the ^{131}I uptake test, as performed in these experiments, is interesting. Stanbury *et al.*[17] have reported that an increase of carrier iodide dose in the uptake test to 1.5 mg does not much influence the uptake of ^{131}I in man, but when the dose exceeds this value, the ^{131}I uptake falls sharply. If we now work in a range where the thyroidal capacity for iodide is already almost completely satisfied because of a plentiful supply of iodine, even very small additions of iodide may influence the form of the ^{131}I accumulation curve. Such a situation is especially likely to occur in communities where large doses (ca. 10 mg) of iodide are supplied at infrequent intervals for the prophylaxis of goitre. The effect on the uptake curve obtained with iodide does not, of course, result in any functional disturbance in the thyroid but is rather to be regarded as a technical error of measurement.

The more intense accumulation of ^{131}I in the thyroid gland of female subjects, combined with the lower total iodine in their blood as compared to males, indicates that a relative iodine deficiency more easily develops in females than in males living in the same circumstances. This is in good accord with the fact that females acquire goitre about five times more frequently than males. Whether this depends on a smaller supply of iodine in the food, a greater iodine requirement, or a less economical utilization of iodine in the body cannot be determined on the basis of the present data. In earlier reports in the literature no such sex difference was remarked[18,19,20].

Summary

Neither milk produced by cattle on an extremely high *Brassica* feed nor milk produced on an ordinary diet could be shown to disturb the accumulation of radio-iodine in the thyroid gland of man.

The quantities of L-5-vinyl-2-thiooxazolidone and thiocyanate required to depress the iodide uptake are higher by a factor of at least 100 than the amounts consumed in milk. Nor was there any evidence of the existence of a hitherto unknown goitrogenic factor in milk.

The thiocyanate content of the serum of smokers was found to be higher by a factor of four than that of non-smokers. No corresponding difference in thyroid function was found.

The method of determining the radio-iodide uptake by the human thyroid gland was demonstrated to be extremely sensitive to fluctuations in iodine supply. Failure to check the iodine balance might be the explanation of earlier reports that a goitrogenic effect has been observed in milk.

The authors wish to express sincere thanks to Professor A. I. Virtanen for his support in this work.

This investigation is a part of a research project under U.S. Public Law No. 480, 83rd Congress.

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